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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	)	
	)	
Hea Young Park Choo et al.	)	
	)	Group Art Unit:1614
Serial No.: 10/789,725	)	
	)	Examiner: SPIVACK, PHYLLIS G
Filed: February 27, 2004	)	

For: METHOD FOR INHIBITING 5-LIPOXYGENASE USING A BENZOXAZOLE DERIVATIVE OR AN ANALOGUE THEREOF

Commissioner for Patents
P. O. Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 C.F.R. §1.132

Sir:

I, Hea Young Park Choo, being a citizen of the Republic of Korea and presently residing at Hyundae Apt. 86-603, Apgujung-dong, Kangnam-gu, Seoul 135-905, Republic of Korea, do declare:

That I am one of the co-inventors of the invention disclosed in the above-identified application, and hence am fully familiar with the subject matter therein; and

That I have conducted an experiment as follows, in order to demonstrate that the compound of formula (I) according to the subject application, which inhibits 5-lipoxygenase is clinically effective in preventing or treating leukotriene-related diseases. A compound of formula (I) (wherein, X is CH, Y is O, n is 1, R^1 is CH_3,

R^2 and R^3 are H, R^4 is C_2H_5, and R^5 is H) having 5-lipoxygenase inhibiting activity of IC_{50}=0.12μM was used as a test compound in the following experiment.

<*In vivo* evaluation of asthma treatment effect by 5-lipoxygenase inhibition >

The *in vivo* efficacy of the test compound for treating asthma was evaluated together with Zileuton (Zyflo™) (Abbott Laboratories), a comparative compound which is a known 5-lipoxygenase inhibitor, as follows.

(1) Mice modeling for sensitization and airway challenge

8 Week old female BALB/c mice were obtained from Korean Research Institute of Chemistry Technology, kept in a laminar flow cabinet and divided into 3 groups (5-6 mice per group). The 3 groups of mice were respectively subjected to the following treatments: (1) sham-sensitization plus challenge with phosphate-buffered saline (PBS; ipNeb); (2) sensitization plus challenge with ovalbumin (OVA) (Sigma A5503; Sigma, St. Louis, MO) (ipNeb); and (3) sensitization with OVA (ip) plus challenge with OVA (Neb) and a drug (the test compound or Zileuton) (po).

Specifically, the test mice were sensitized with intraperitoneal injection of 20 μg OVA with 4 mg of adjuvant aluminum hydroxide on days 0 and 11. The mice were challenged through the airways with OVA (1% in PBS) on day 11, 21, 22, 23 and 25 after the initial sensitization to induce inflammation. A 50mg/kg bodyweight dosage of a test drug was orally administered once a day on days 21-25. The mice were assessed 24 hours after the last challenge for the suppressive effect of the drug on the airways of allergic asthma.

(2) Determination of Airway Hyperresponsiveness

Airway hyperresponsiveness (AHR) was determined 24 hours after the final challenge. Each mouse was placed in a barometric plethysmographic chamber and challenged with aerosolized PBS for 3 min, followed by challenging with increasing concentrations of aerosolized methacholine from 0 to 30 mg/ml, at intervals of 5 mg/ml, each for 3 min, and AHR was recorded for 5 minutes

thereafter. To evaluate the degree of AHR, the enhanced pause Penh values of the drug sample was obtained during each methacholine challenge, and expressed as a percentage of a basal Penh value obtained in control (PBS) challenge, in which a Penh value was calculated as follows:

$$Penh = [T_e /(RT-1)] \times [PEF / PIF]$$

wherein, T_e is expiratory time; RT is relaxation time; PEF is peak expiratory flow; and PIF is peak inspiratory flow.

The results are shown in Table I.

Table I

Methacholine Concentration	0mg/ml	5mg/ml	10mg/ml	20mg/ml	30mg/ml
Control (OVA only)	0.656	1.376	2.418	2.878	3.277
Zileuton	0.567	1.673	2.344	2.588	2.866
Inventive Compound	0.383	0.756	1.339	1.863	2.724

As shown in Table I, the inventive compound exhibited significantly low hyperresponsiveness.

(3) Measurement of IL-4, IL-5 and IL-13 level

The mice were sacrificed by pentobarbital overdose (Sigma P3761) 24 hours after AHR measurement and tracheotomy was performed. After ice-cold PBS (0.5 ml) was introduced into the lung, bronchoalveolar lavage fluid (BALF) was obtained by aspiration three times (total 1.5 ml) *via* tracheal cannulation. BALF was centrifuged at 4℃, and the supernatant was collected and stored at –70°C until use. The amount of cytokines IL-4, IL-5 and IL-13 in BALF was measured by a specific mouse ELISA kit (R&D Systems; Minneapolis, MN) and the results are shown in Tables II to IV.

Table II

IL-4 Amount (ng/ml) in BALF							
	1	2	3	4	5	a.v.[1)]	s.d.[2)]
Control (OVA only)	422.03	476.73	316.4	424.01	470.2	421.872	64.1715
Zileuton	274.43	321.74	358.3	266.8	204.9	285.234	58.2696
Inventive Compound	147.6	234.42	218.2	154.17	237.3	198.34	43.9897
1) Average value 2) Standard deviation							

Table III

IL-5 Amount (ng/ml) in BALF							
	1	2	3	4	5	a.v.[1)]	s.d.[2)]
Control (OVA only)	248.82	227.21	219.52	215.05	323	246.717	44.5694
Zileuton	113.31	161.01	181.44	219.27	214.3	177.864	43.3198
Inventive Compound	174.05	109.83	112.81	86.353	101.6	116.923	33.5457
1) Average value 2) Standard deviation							

Table IV

IL-13 Amount (ng/ml) in BALF							
	1	2	3	4	5	a.v.[1)]	s.d.[2)]
Control (OVA only)	128.45	123.16	105.09	191.87	165.9	142.89	35.1914
Zileuton	124.37	101.3	83.84	85.45	104.3	99.842	16.4802
Inventive Compound	75.23	48.19	41.873	80.2	76.02	64.303	17.8335
1) Average value 2) Standard deviation							

As shown in Tables II to IV, the inventive compound exhibited the improved

inhibiting activity for the formation of cytokines IL-4, IL-5 and IL-13 in BALF.

(4) Histopathology studies

The lung tissue from each sacrificed mouse was fixed in 10% neutral-buffered formalin for 20 to 24 hours, embedded in paraffin, sliced into 4 □ thickness sections, and stained with H-E solution (hematoxylin; Sigma MHS-16 and eosin, Sigma HT110-1-32). Subsequently, the stained tissue was mounted and cover-slipped with Dako-mounting medium (Dakocytomation; Denmark Carpinteria CA). The degree of inflammatory cell infiltration in lung sections, specifically the degree of peri-bronchiole and peri-vascular inflammation was evaluated by a specific standard scale, i.e., scoring with 0–3 (0, no inflammatory cell; 1, few inflammatory populations; 2, a thin ring of inflammatory cells (one to five cell-layer deep); 3, a thick ring of inflammatory cells (more than five cell-layer deep) and averaged. The results are shown in Table V.

Table V

Inflammation Score	Control (OVA only)	Zileuton	Inventive Compound
Average value	2.225	1.835	1.2427
Standard deviation	0.224	0.195	0.232

As shown in Table V, the inventive compound has good suppressive effect on the leukocite infiltration.

Furthermore, there are numerous reports such as Exhibits 1 to 11, as attached to Amendment and Request for Reconsideration under 37 C.F.R. §1.111, showing the correlation between 5-lipoxygenase and the diseases of claim 2, and it is well known in the art that such diseases can be prevented or treated by inhibiting 5-lipoxygenase.

Accordingly, I believe that the inventive compound inhibiting 5-lipoxygenase is also effective in preventing or treating leukotriene-related diseases such as pertussis, psoriasis, rheumatic arthritis, arthritis, inflammatory bowel disease, cystic fibrosis, acute/chronic bronchitis, sepsis, cardiac myoischemia, cardiac anaphylaxis, ischemia and allergic rhinitis, as well as asthma.

I hereby declare that all statements made herein of my own knowledge are true and all statements made on information and belief are believed to be true; and, further, that these statements made are punishable by fine or imprisonment, or

both, under section 1001 of Title 18 of Unites States Code and that such willful false statements may jeopardize the validity of the application or any patents issuing thereon.

Further the deponent saith not.

Date: 27th day of September, 2006

Hea Young Choo

(Hea Young Park Choo)